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Lipophilic platinum complexes entrapped in liposomes: improved stability and preserved antitumor activity with complexes containing linear alkyl carboxylato leaving groups

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Abstract. Lipophilic diaminocyclohexane (DACH) platinum complexes have shown significant promise in preclinical studies. One of these compounds, *cis*-bis-neodecanoato-*trans*-R,R-1,2-diaminocyclohexaneplatinum(II) (NDDP), which contains two branched leaving groups of 10 carbons, showed a favorable toxicity profile in a liposomal formulation in early clinical trials. However, like many other DACH platinum compounds with branched leaving groups, it is unstable within the liposomes, thus preventing its widespread clinical evaluation. We studied the effect of the configuration of leaving groups on intraliposomal complex stability by studying a series of DACH platinum complexes containing linear alkyl carboxylato leaving groups of 5–18 carbons. The entrapment efficiency was greater than 90% for all liposomal preparations of the complexes and was independent of lipid composition and length of the leaving group. The drug leakage from the liposomes was minimal, but was directly related to the length of the leaving group. Intraliposomal stability was inversely related to the length of the leaving group and the content of DMPG (dimyristoyl phosphatidylglycerol) in the liposomes. The effect of length of leaving group on intraliposomal stability was minimal in compounds with leaving groups smaller than 10 carbons, but very pronounced in compounds with longer leaving groups. Stable liposomal formulations of selected compounds with leaving groups of 6 and 10 carbons had significant in vivo antitumor activity against both L1210/S and L1210/PDD leukemias. The results indicate (1) that compounds with linear leaving groups are much more stable within DMPG-containing liposomes than compounds with branched leaving groups and (2) that DMPG is required for in vivo antitumor activity. Stable and active liposomal formulations of selected compounds with linear leaving groups have been identified. These formulations are candidates for clinical development.

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Abbreviations: DMPC, dimyristoyl phosphatidylcholine; DMPG, dimyristoyl phosphatidylglycerol; L-NDDP, liposomal *cis*-bis-neodecanoato-*trans*-1,2-diaminocyclohexaneplatinum(II).

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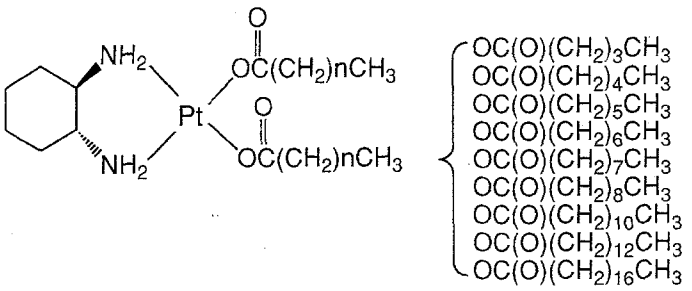
Introduction

cis-Diamminedichloroplatinum(II) (cisplatin) is effective in the treatment of various types of human cancer [13]. However, its use is limited by significant toxic side effects, such as acute nephrotoxicity and chronic neurotoxicity, and natural or acquired tumor drug resistance.

In an attempt to overcome these limitations, during the last few years we have synthesized and tested new lipophilic platinum(II) complexes that can be entrapped in liposomes for their i.v. administration [7, 10–12, 15]. The general structure of these complexes is [DACH-Pt-R_2], where DACH is 1,2-diaminocyclohexane and R is a lipophilic carboxylato group. The leading compound used in our preclinical and early clinical trials was NDDP [*cis*-bis-neodecanoato-*trans*-R,R-1,2-diaminocyclohexaneplatinum (II)] entrapped in multilamellar vesicles composed of dimyristoyl phosphatidylcholine (DMPC) and dimyristoyl phosphatidylglycerol (DMPG). Liposomal NDDP (L-NDDP) was found to be not cross-resistant with cisplatin in vitro and in vivo [10], non-nephrotoxic in mice and dogs [11] and more active against experimental in vivo models of liver metastases [10]. Its ability to overcome cisplatin resistance was associated with a similar cellular drug accumulation and DNA platination in both sensitive and resistant cells [5]. In a phase I study, L-NDDP was non-nephrotoxic, and its limiting toxicity was myelosuppression [12].

L-NDDP has two major limitations. First, NDDP is a mixture of 15–20 isomers, thus making its full chemical



Carbon No. of Leaving Group
5
6
7
8
9
10
12
14
18

Fig. 1. Chemical structure of lipophilic diaminocyclohexane platinum (II) complexes with linear alkyl carboxylayto ligands as leaving groups. The number of carbons in the leaving group corresponds to the abbreviated number of the platinum complex in this paper.

characterization difficult, if not impossible. Second, NDDP undergoes a chemical degradation/activation process into one or more active intermediates within the liposomes shortly after liposome preparation. This chemical degradation process is dependent on the content of DMPG in the lipid bilayer and is essential for the complex to exert its antitumor activity in vivo. As a result, L-NDDP does not meet standard chemical stability criteria for a pharmaceutical product.

To evaluate this class of agents in humans fully and appropriately, there is a need for the development of effective formulations that display antitumor activity while preserving the chemical stability of the platinum complex within the liposomes before administration to the patient. For that purpose, we are conducting studies to improve understanding of the chemical interaction between these complexes and the lipid components of the liposomes. As a first step, we decided to investigate whether the intraliposomal stability of these complexes depends on the spatial configuration of the leaving group. New highly lipid-soluble *cis*-bis-carboxylato-*trans*-1,2-diaminocyclohexaneplatinum(II) complexes containing linear aliphatic carboxylato ligands as leaving groups instead of branched and cyclic carboxylato groups were synthesized [1]. Different liposomal formulations of these compounds were prepared and tested for entrapment efficiency, drug stability, and in vivo antitumor activity against L1210/S and L1210/PDD leukemias under fixed ex vivo conditions. Our results demonstrate that the configuration of the leaving group is an essential determinant of the intraliposomal stability of these compounds: compounds with linear leaving groups are much more stable in DMPG-containing liposomes than are compounds with branched leaving groups while displaying similar antitumor activity. Liposomal formulations of some of the compounds may meet stability criteria of a pharmaceutical product and may, therefore, be candidates for future development.

Materials and methods

Platinum(II) complexes and chemicals. A series of *cis*-bis-carboxylato-*trans*-1,2-diaminocyclohexaneplatinum(II) complexes was prepared by the reactions of 1,2-diaminocyclohexane-diiodideplatinum(II) with the silver salt of corresponding carboxylic acid, and the complexes were purified by recrystallization from acetone, as previously reported [1] (shown below).

$$[(DACH)PtI_2] + 2[AgOCOR] \rightarrow [(DACH)Pt(OCOR)_2] + 2\,AgI$$

The structure of the compounds was confirmed by elemental analysis and by ^{1}H, ^{13}C, ^{195}Pt nuclear magnetic resonance (NMR) and infrared spectroscopy. ^{195}Pt NMR showed the corresponding peaks in the range of −1713 to −1775 ppm in chloroform solution. These peaks were measured relative to an external standard of 2.2 M Na_2PtCl_6 in D_2O at 0.0 ppm. The chemical structures of all platinum complexes are shown in Fig. 1. The linear leaving groups used were pentanoato (*5*), hexanoato (*6*), heptanoato (*7*), octanoato (*8*), nonanoato (*9*), decanoato (*10*), laurato (*12*), myristato (*14*), and stearato (*18*) carboxylate. The molecular weights are 511, 539, 567, 595, 623, 651, 707, 763, 876 for *5*, *6*, *7*, *8*, *9*, *10*, *12*, *14*, *18*, respectively. Compounds are abbreviated with the number of carbons in the leaving group.

All these complexes are highly soluble in chloroform and other organic solvents, but they are insoluble in water. Chromatographically pure DMPC and DMPG were obtained from Avanti Polar Lipids (Birmingham, Ala) and used without further purification. Methyl and *t*-butyl alcohol were purchased from Sigma (St. Louis, Mo.).

Instruments. High-performance liquid chromatography (HPLC) analyses were conducted with a Waters Associates unit: 510 pump, Model 80 gradient controller, 712 WISA automatic sampler, 481 LC detector, and 3390A integrator (Hewlett Packard, Pa.). The samples were eluted through a Chromega-8 bond column (ES Industries, N. J.) using aqueous methanol as an eluant. The solvents were filtered (Millipore FH or GVWP, 0.45 mm) and degassed prior to use. The flow rate was 1.0 ml/min, and the samples were detected by UV detector at 224 nm. The HPLC retention times (min) for the platinum complexes and lipids are as follows: *5* (4.03), *6*, (5.32), *7* (7.62) using 20% H_2O-methanol; *8* (5.30), *9* (6.71), DMPC and DMPG (2.81) using 10% H_2O-methanol; *10* (4.70), *12* (6.10) using 5% H_2O-methanol, *14* (4.93), *18* (7.30) using 100% methanol as the eluant. Thin-layer chromatography (TLC) was run on precoated silica gel GHLF microscope slides (2.5×10 cm; Analtech no. 21521, DE). The eluant system employed was 10% methanol-chloroform for normal-phase TLC. The R_f values for the compounds are as follows: *5* (0.22), *6* (0.30), *7* (0.34), *8* (0.43), *9* (0.50), *10* (0.52), *12* (0.52), *14* (0.55), *18* (0.52), DMPC (0.07), and DMPG (0.14). All the platinum complexes were found to be >95% pure by TLC before use.

Atomic absorption spectra were recorded with the following Varian Techtron units: SpectrAA-30 Model atomic absorption spectrophotometer (AAS) at 265.9 nm with platinum hollow cathode lamp (No. 56-101041-00), GTA-96 graphite tube analyzer, and DS-15 data station. The platinum concentrations in the samples were determined from a calibration curve prepared with Na_2PtCl_6 solutions in 0.1 *N* HCl solution.

Preparation of liposomes containing platinum complexes. Multilamellar vesicles containing the different complexes (*5*, *6*, *7*, *8*, *9*, *10*, *12*, *14*, and *18*) were prepared as previously described [7, 11, 12]. In brief, chloroform solutions of each lipid either using only DMPC or using a 7:3 or 3:7 molar ratio of DMPC:DMPG were mixed and the chloroform was removed in a rotary evaporator at 40° C under reduced pressure. To the dried lipid film, tert-butanol solutions of different drugs were added for a final drug:lipid weight ratio of 1:15, and the solutions were shaken slowly at 40° C for 5–10 min. The solutions

were aliquoted in vials containing 5 mg of each drug and frozen in a dry ice-acetone bath. tert-Butanol was removed by lyophilization (freeze dryer model 8, LabConco) overnight to give a white lipid powder containing 5 mg drug and 75 mg lipids.

Determination of entrapment efficiency. The lyophilized drug: lipid powders in each vial were reconstituted with 5 ml of saline by vigorous manual shaking for 5–10 min (final concentration: 1 mg/ml). All preparations were initially checked by light microscopy to rule out the presence of unentrapped, precipitated drug. Subsequently, the liposomal suspension was centrifuged at 20,000 rpm for 1 h and a sample of supernatant was taken. The presence of platinum species in the supernatant was analyzed by determining the amount of elemental platinum by AAS. The percent entrapment efficiency (% EE) was calculated as:

$$\%\,EE = \frac{\text{Total platinum complex (T)} - \text{Platinum complex in supernatant (S)}}{\text{Total platinum complex (T)}} \times 100$$

Total platinum complex initially added (T) was determined after 1:10 dilution of the original liposome suspension with methanol. Both solutions were diluted in 0.1 *N* HCl prior to platinum determination. The percentage of drug leakage into the aqueous phase was calculated as:

$$\%\text{ of drug leakage} = \%\,EE_{0h} - \%\,EE_{6h}.$$

All experiments were performed in duplicate.

The size distribution of the different liposomal preparations was determined in a Coulter counter (Hialeah, Fla.).

Intraliposomal drug stability. The stability of drugs within the liposomes was determined by comparing the HPLC profiles as a function of time. First, liposome-entrapped drugs were prepared in 0.9% NaCl solution and kept at 25°C. Liposome suspension aliquots of 0.1 ml were diluted in 0.9 ml of methanol at 0, 2, and 6 h after liposome formation, and the drug integrity was analyzed immediately by HPLC, as described above. In order to quantitate the amount of drug from the HPLC profiles, the integrated areas of the drug peaks at 224 nm were normalized using a standard curve prepared with different concentrations of NDDP in methanol. The percentage of drug stability was calculated as:

$$\%\text{ of drug stability}_{(x)h} = \frac{\text{Amount of drug detected at } (\times)\text{ h}}{\text{Amount of drug initially added}} \times 100$$

All these experiments were performed in triplicate.

Antitumor activity. Antitumor activity experiments were carried out in B6D2F1 mice inoculated with L1210 leukemia cell lines sensitive (L1210/S) or resistant (L1210/PDD) to cisplatin. The objective of these studies was to discard the inactive preparations, not to select the most active one. Mice were purchased from Harlan (Houston, Tex.), and the cell lines were obtained from the Tumor Repository, National Cancer Institute (Bethesda, Md.). Groups of 6–8 animals were used. Tumor cells (1×10^6 cells in 0.2 ml saline) were inoculated i.p. on day 0. Treatment was started i.p. on day 1, and the injection volume of each liposomal drug ranged between 0.2 to 0.5 ml. Treatment consisted of a single i.p. injection on day 1 for the L1210/S model and three i.p. injections on days 1, 5, and 9 for the L1210/PDD model. The results were expressed as:

$$\%\,T/C = \frac{\text{Median survival of treated animals}}{\text{Median survival of untreated animals}} \times 100$$

Two independent experiments were performed.

Results

Entrapment efficiency

Table 1 shows percent entrapment efficiency at 0 and 6 h after the preparation of different formulations of the platinum compounds. The complexes were formulated in liposomes containing DMPC:DMPG at 7:3 and 3:7 molar ratios. The %EE of most liposomal platinum formulations was very high at 0 h (range 92.1–96.0%), whether the 7:3 or 3:7 DMPC:DMPG lipid ratio was used and was not significantly affected by the number of carbons in the linear leaving groups. No crystals of free drug were observed in any of the preparations as assessed by optic microscopy. The median size of all liposomal preparations was 2–3 μm. Drug leakage was minimal but directly related to the length of the leaving groups and relative amount of DMPG in the liposomes. For example, the drug leakage over time for L-*6*, L-*10*, and L-*14* was 0.9, 1.2, and 5.1% for the 7:3 lipid ratio and 0.4, 5.0, and 13.0% for the 3:7 lipid ratio, respectively.

Table 1. Entrapment of platinum compounds in liposomes and drug leakage at 6 h

No carbons leaving group	Drug entrapment (% EE)		Drug leakage (%)	
	DMPC:DMPG 7:3	DMPC:DMPG 3:7	DMPC:DMPG 7:3	DMPC:DMPG 3:7
5	92.9	93.2	0.5	1.2
6	96.0	95.4	0.9	0.4
7	92.1	92.8	1.1	2.0
8	94.8	94.7	1.4	3.4
9	92.9	94.1	2.5	3.6
10	93.2	92.3	1.2	5.0
12	94.7	93.9	4.2	6.0
14	94.0	94.6	5.1	13.0
18	95.2	92.5	7.9	12.3

EE, Entrapment efficiency; DMPC, dimyristoyl phosphatidylcholine; DMPG, dimyristoyl phosphatidylglycerol. Values are all means of two separate experiments with a standard error $<\pm3\%$. Drug leakage calculated as: ËE at 0 h – ËE at 6 h

Intraliposomal drug stability

The stability of the liposomal platinum(II) analogues was monitored by HPLC at 0, 2, and 6 h after preparation of the liposome formulations, and the integrated area of the drug detected in the HPLC chromatogram was converted into the percentage of drug remaining at the time tested. The average data from three independent experiments are shown in Table 2. L-*5*, -*6* and -*7* were stable in liposomes when either 7:3 or 3:7 DMPC:DMPG lipid ratios were used. L-*8*, -*9*, -*10*, and -*12* were stable in preparations with a 7:3 lipid ratio with only traces of degradation, but the same drugs displayed significant degradation (10–20%) in the preparation with a 3:7 ratio at 6 h. In the case of L-*14* and -*18*, significant degradation occurred with either the 7:3 or 3:7 lipid ratio preparations (30–50% in the 7:3 formulation and 65–72% in the 3:7 formulation at 6 h). These results indicate that drug stability is inversely related to the amount of DMPG in the liposomes and the number of carbons in the leaving groups. The 7:3 lipid ratio composition resulted in significant degradation of compounds *14* and *18* (25–50%), a lesser degree of degradation of compounds *8*, *9*, *10*, and *12* (0–10%), and none of compounds *5* and *6*. The 3:7 lipid ratio formulations resulted in sig-

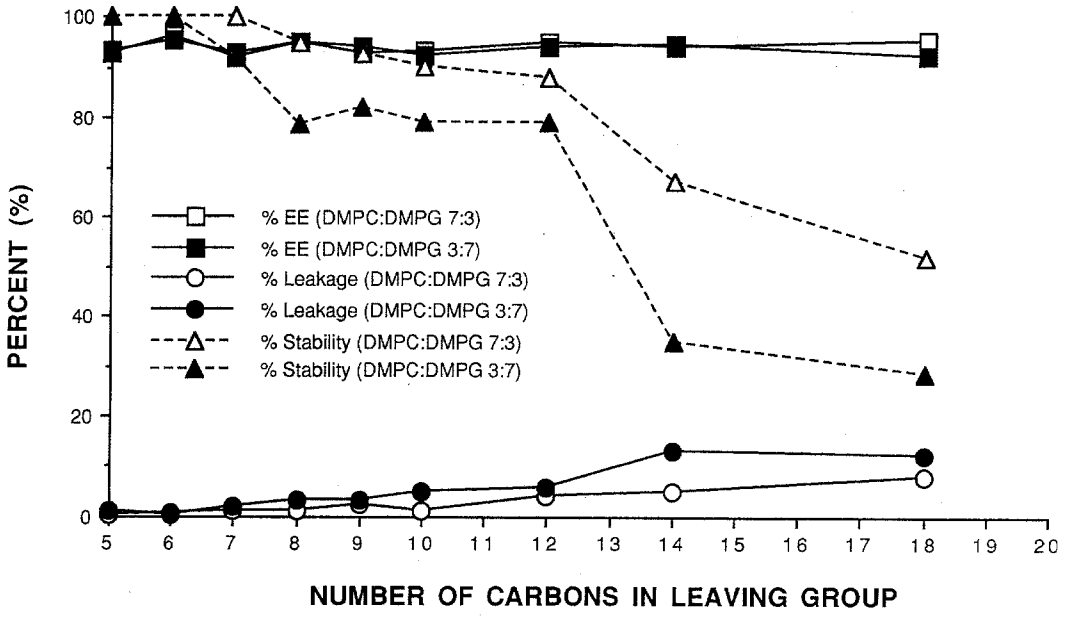


Fig. 2. Relationship between drug entrapment (□), drug leakage (○), intraliposomal drug stability (△), content of DMPG (*white symbols*, DMPC:DMPG 7:3; *dark symbols*, DMPC:DMPG 3:7) and number of carbons in the leaving group

nificant degradation (22–72%) of most compounds tested except for compounds *5*, *6*, and *7* (0–8%).

The degradation products were not detected as new peaks in the HPLC chromatogram, maybe because they overlap with the DMPC or DMPG peaks. Normal-phase TLC (10% methanol-chloroform) of these solutions showed several spots of byproducts below the starting materials (R_f = 0.55 and 0.52 for *14* and *18*, respectively) as a long tail (R_f = 0.20~0.48).

The relationship among entrapment efficiency, drug leakage, and drug stability was plotted (Fig. 2). Although the entrapment efficiency was not affected by the length of the leaving group, the drug leakage increased and drug stability decreased with increasing number of carbon atoms in the carboxylate leaving group; however, these effects were not parallel for compounds with more than 12 carbons. The degradation of compounds with more than 12 carbons was 65–72% with the 3:7 lipid ratio composition; however, the entrapment efficiency remained high, 80–82%, as was the case for the other compounds. These results indicate that most of the degradation products remain entrapped within the lipid bilayer.

Table 2. Intraliposomal stability of platinum compounds

No. of carbons leaving group	DMPC:DMPG (molar ratio)	% Stability		
		0 h	2 h	6 h
5	7:3	100	100	100
	3:7	100	100	100
6	7:3	100	100	100
	3:7	100	100	100
7	7:3	100	100	100
	3:7	100	100	91.8±3.4
8	7:3	100	95.0±1.8	94.8±2.0
	3:7	100	93.5±2.3	78.8±3.8
9	7:3	100	92.6±1.5	92.6±1.7
	3:7	100	90.6±3.4	82.2±5.1
10	7:3	100	95.0±2.2	90.1±2.5
	3:7	100	91.0±3.0	79.0±4.9
12	7:3	100	95.0±3.1	88.2±2.9
	3:7	100	89.0±4.4	79.0±4.9
14	7:3	100	77.7±4.5	67.1±3.5
	3:7	83.5±4.7	38.1±7.7	35.0±6.3
18	7:3	100	67.5±7.3	51.8±3.9
	3:7	56.0±5.1	29.0±8.8	28.3±9.4

Means of three independent experiments. Drug stability assessed by HPLC as described in Materials and methods

Antitumor activity

Liposomal formulations of complexes with leaving groups of 6, 10, and 14 carbons were tested and compared with cisplatin. L-*6*, L-*10*, and L-*14* were selected to investigate the correlation between drug stability and antitumor activity. Compound *6* does not display degradation in either lipid ratio composition, *10* displays slight degradation (10%), and *14* is highly unstable in either lipid composition (30–65% degradation). In the first set of experiments, the effects of a single i.p. dose of cisplatin, L-*6*, -*10*, and -*14* using DMPC:DMPG ratios of 7:3, 3:7, and DMPC alone as lipid compositions were tested against L1210/S leukemia. BDF_1 mice were inoculated i.p. on day 0 with 1×10^6 cells. Treatment was given i.p. on day 1 only. The doses of liposomal platinum were 12.5, 25, 50, and 100 mg/kg (platinum complex/mouse). Table 3 shows the result of antitumor activity of L-*6*, -*10*, and -*14* against L1210/S leukemia. The %T/C values are the mean values from duplicate experiments.

For the 7:3 lipid ratio composition, the optimal dose of L-*6*, -*10*, and -*14* was 100 mg/kg. The %T/C at these doses was 171, 200, and 175, respectively. For the 3:7 lipid ratio composition, the optimal dose of L-*6*, -*10*, and -*14* was 100, 75, and 50 mg/kg, respectively. The %T/C at these doses was 185, 214, and 162, respectively. The optimal dose of the compounds tested decreased with increasing the DMPG content in the lipid bilayer. A higher relative content of

Table 3. In vivo antitumor activity against L1210/S leukemia of liposomal preparations of compounds with 6, 10, and 14 carbons

Compound	Dose mg/kg	%T/C		
		DMPC:DMPG 7:3	DMPC:DMPG 3:7	DMPC only
L-6	12.5	114	128	
	25.0	128	128	
	50.0	142	157	
	75.0	157	171	128
	100.0	171	185	
L-10	12.5	128	142	
	25.0	142	–	
	50.0	171	171	
	75.0	186	214	128
	100.0	200	185	
L-14	12.5	111	150	
	25.0	150	162	
	50.0	162	162	
	100.0	175	Toxic	
Cisplatin	10.0	162–185		

Tumor inoculation (1×10^6 cells i.p.) on day 0. Treatment i.p. on day 1. Values are means of duplicate experiments

Table 4. In vivo antitumor activity against L1210/PDD leukemia of liposomal preparations of compounds with 6 and 10 carbons

Compound	Dose mg/kg	%T/C	
		DMPC:DMPG 7:3	DMPC:DMPG 3:7
L-6	100×1	155	163
	30×3	181	200
	50×3	190	Toxic
L-10	75×1	190	163
	30×3	190	212
	50×3	200	Toxic
Cisplatin	10×1	109	
	6×3	118	

Tumor inoculation (1×10^6 cells i.p.) on day 0. Treatment i.p. on day 1, or days 1, 5, and 9. Values are means of duplicate experiments

DMPG was accociated with a slightly increased antitumor potency for all compounds tested. At the doses studied, the compound that displayed higher antitumor activity was L-*10*, with %T/C of 200–214 independently of DMPG content. This %T/C was higher than that obtained with cisplatin (162–185). L-*6* and -*10* liposomal formulations composed of DMPC alone were devoid of antitumor activity (%T/C = 128), thus indicating that the presence of DMPG is essential for in vivo biological activity.

From these results, the most interesting finding is that, although L-*6*, and L-*10* are highly stable in 0.9% NaCl solution, with either the 7:3 or the 3:7 lipid ratio composition for a period of up to 6 h, they have significant antitumor activity against L1210/S leukemia. This indicates that no active intermediates need to form within the liposomes prior to administration for these compounds to exert their in vivo biological activity.

Table 4 shows the antitumor activity of L-*6*, -*10*, and -*14* using different relative contents of DMPG against L1210/PDD leukemia. Treatment was administered i.p. on day 1 only or on days 1, 5, and 9. For the 7:3 lipid ratio formulations, the optimal dose of L-*6* and -*10* was 50 mg/kg on days 1, 5 and 9. The %T/C at these doses was 190 and 200, respectively. For the 3:7 lipid ratio formulations, the optimal dose of L-*6* and -*10* was 30 mg/kg on days 1, 5, and 9. %T/C at these doses was 200 and 212, respectively. Therefore, the amount of DMPG in the liposomes had little effect on the level of antitumor activity but increased somewhat the drug potency. The highest level of antitumor activity was observed with L-*10* composed of DMPC:DMPG = 3:7 (%T/C:212). As expected, cisplatin was devoid of antitumor activity against this tumor model (%T/C: 109–118).

Discussion

Work reported by us [5] and by other investigators [4, 8] has consistently indicated that lipophilic platinum complexes are promising therapeutic agents that remain to be appropriately tested in the clinical setting. A particularly interesting potential property of these agents is their ability to overcome cellular drug resistance associated with reduced cellular accumulation of cisplatin by displaying a markedly enhanced cellular transmembrane transport. The DACH platinum complexes have attracted significant attention for many years because they are not cross-resistant with cisplatin [2], probably as a result of the induction of DNA-Pt adducts that are poorly repaired in resistant cells although the DNA adducts they form are identical to those of cisplatin [6], or inhibition of essential processes, such as replication or transcription [14]. The basic strategy of our program was to design highly lipophilic DACH compounds that would display lack of cross-resistance as a result of the two mechanisms mentioned above and to select, among those, complexes compatible with liposome carriers. This approach, we hypothesized, would allow us to avoid the use of detergents for the compounds' i.v. administration and to alter their pharmacokinetics and organ distribution by using lipid vesicles with different physico-chemical properties.

In spite of encouraging results in preclinical and early clinical studies with our leading complex, L-NDDP [7, 10–12], we soon discovered that, to exert its antitumor effect, NDDP had to react with DMPG within the liposome membranes prior to i.v. administration [9]. In accordance with this finding, free NDDP was devoid of antitumor activity. As a result, a broad phase II testing program had to be deferred until a better understanding of the chemical reactions between the lipids and NDDP was sorted out. Extensive ongoing characterization efforts suggest that DACH-Pt-DMPG complexes are formed early on and might be the active or one of the active intermediates.

In the current study, we examined the effect of the structure of leaving group of platinum(II) complexes on drug stability and antitumor activity. The results provide definite evidence that the spatial configuration of the leaving group plays a crucial role in the reactivity of these complexes within the liposomes as well as affecting their

liposome entrapment and leakage. Liposomal preparations of a series of lipophilic platinum complexes containing linear carboxylate groups of 5 to 18 carbon atoms as leaving groups were prepared using DMPC and DMPG as lipid components of the liposomes. The entrapment efficiency was very high (>90%) and independent of the lipid composition and length of the leaving group. No crystals of free drug were observed. Leakage of platinum species from the liposomes to the aqueous phase at 6 h was minimal but directly related to the length of the leaving group (0.5–1.2% for the compound with leaving groups of 5 carbons and 7.9–12.3% for the compound with leaving groups of 18 carbons). It was also enhanced by the relative content of DMPG in the lipid bilayers. With compounds with branched leaving groups we found opposite results: entrapment efficiency and drug leakage were directly and inversely related, respectively, to size of the leaving groups [9].

The intraliposomal stability of complexes with linear leaving groups was much higher than that reported by us with complexes with branched leaving groups [9]. As in the case of compounds with branched leaving groups, stability was inversely related to length or size of leaving group and content of DMPG. However, in contrast with the complexes with branched leaving groups, these factors appeared to be of little impact for compounds with linear leaving groups of 10 carbons or fewer. The platinum(II) complexes with leaving groups of 5 or 6 carbons were completely stable within the lipid bilayers (100% at 6 h), independently of the presence of DMPG. Complexes with carboxylate leaving groups of 7–10 carbons were also very stable (≥95% at 6 h) in liposomes composed of DMPC:DMPG at a 7:3 molar ratio. This contrasts with a stability of about 40% for NDDP (branched leaving groups of 10 carbons) entrapped in liposomes of the same composition [9]. Complexes with leaving groups of 12, 14, and 18 carbons were unstable in either liposomal formulation, whether the relative DMPG content was low or high.

Our most interesting finding is that complexes with linear leaving groups of 10 or fewer carbons are not only much more stable than compounds with branched leaving groups within the liposomes, but also do not need to react with the lipids prior to in vivo administration to exert their antitumor activity as in the case of NDDP. The complex with a leaving group of 6 carbons exhibited in vivo antitumor activity similar to or greater than that of cisplatin against L1210/S and also had significant in vivo antitumor activity against L1210/PDD in formulations with 100% drug stability. The complex with leaving groups of 10 carbons displayed in vivo antitumor activity similar to or slightly higher than that of the complex with leaving groups of 6 carbons in liposomal formulations with over 90% drug stability. In a previous study, we have already demonstrated that this compound has significant antitumor activity by the i.v. route [7].

The practical consequence of this finding is that stable and active liposomal formulations with some of these compounds are possible (short linear leaving groups). Still, as in the case of NDDP, an intriguing finding was that the presence of DMPG appeared to be an essential prerequisite for antitumor activity in spite of not resulting in degradation/activation of the compounds within the liposomes, since formulations composed of DMPC alone were stable but inactive. In in vitro studies, the presence of DMPG enhances two-fold the cellular drug uptake in both sensitive and resistant cells and appears also to enhance the drug cytotoxicity, but only when short drug incubation times are used [5]. It is, therefore, unlikely that the drastic differences between formulations containing DMPC alone and DMPC:DMPG combinations may be explained exclusively by a two-fold enhancement of cellular drug uptake. A simple but likely explanation is that liposomes composed of DMPC alone tend to aggregate very quickly as a result of lack of electrical charge on their surface. As a result, their distribution in the peritoneal cavity may be very uneven, thus not reaching all tumor cells.

In summary, our findings suggest that liposome formulations of platinum complexes with linear leaving groups of 5–10 carbons meet standard stability criteria for pharmaceutical products. Current studies are aimed at assessing the effect of other formulation variables such as pH, temperature, lipid composition, and liposome size on the stability and activity of these compounds. As shown by other investigators [3], tumor targeting of liposome-entrapped drugs can be enhanced by changing the composition and size of the liposomes. The final goal of all these formulation studies is to select a formulation and compound for further preclinical and clinical development.

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